

CLAIMS

1. Use of nucleotide sequences corresponding to the *SILVER* gene, also designated *SI* gene, and to the different allelic forms of this gene, or corresponding to fragments of this gene or of its different allelic forms, for the implementation of a method for the identification of different populations or breeds of ruminant mammals such as cattle, sheep and goats.

2. Use according to claim 1, of nucleotide sequences corresponding to the bovine *SILVER* gene represented by SEQ ID NO: 1, coding for the bovine SI protein represented by SEQ ID NO: 2, and to the different allelic forms of this gene, or corresponding to fragments of this gene or of its different allelic forms, for the implementation of a method for the identification of different cattle populations or breeds, or of different cattle herds each including several cattle populations or breeds.

3. Use of nucleotide sequences according to claim 1 or 2, for the implementation of a method making it possible to check, or even to certify, that an animal belongs to a particular cattle population or breed, or to a particular cattle herd, or, on the other hand, making it possible to certify the exclusion of this animal from this population or breed, or from this particular herd.

4. Use of nucleotide sequences according to one of claims 1 to 3, characterized in that cattle populations or breeds or cattle herds are of French origin.

5. Use according to one of claims 1 to 4,
- of the nucleotide sequence corresponding to the *si* allelic form represented by SEQ ID NO: 3, coding for the bovine si protein represented by SEQ ID NO: 4, or corresponding to fragments of this allelic form, said allelic form comprising the G93A mutation with respect to the *SI* gene, for the implementation of a method making it possible to check, or even to certify, that an animal belongs to the Charolais breed,

- of the nucleotide sequence corresponding to the *si_l* allelic form, represented by SEQ ID NO: 5, coding for the bovine *si_l* protein represented by SEQ ID NO: 6, or corresponding to fragments of this allelic form, said allelic form comprising a deletion

of the three nucleotides TTC situated in positions 82, 83 and 84 with respect to the *SI* gene, for the implementation of a method making it possible to check, or even to certify, that an animal belongs to the Simmental breed,

- of the nucleotide sequence corresponding to the bovine *SI* gene represented by
 5 SEQ ID NO: 1, or to fragments of this gene, for the implementation of a method making it possible to certify the exclusion of an animal from the Charolais breed.

6. Use according to one of claims 1 to 5, of fragments of the nucleotide sequences corresponding to the allelic forms, *SI*, *si*, and *si₁*, represented by
 10 SEQ ID NO: 1, 3, and 5 respectively, said fragments being chosen from those of approximately 10 to 300 nucleotides containing the nucleotides situated in positions 82 to 93 of said sequences.

7. Use according to one of claims 1 to 6, of fragments of the nucleotide
 15 sequences corresponding to the allelic forms *SI*, *si*, and *si₁*, said fragments being chosen from those of 294 nucleotides delimited by the nucleotides situated in positions 9 and 302 of the sequences SEQ ID NO: 1 and 3, these fragments being represented by the sequences SEQ ID NO: 7, and SEQ ID NO: 8 respectively, and the fragment of 291
 20 nucleotides delimited by the nucleotides situated in positions 9 and 299 of the sequence SEQ ID NO: 5, this fragment being represented by the sequence SEQ ID NO: 9.

8. Use of nucleotide primers making it possible to amplify the number of copies of the SILVER gene, or of the different allelic forms of this gene, or of the fragments of this gene or of its different allelic forms, as defined in one of claims 1 to 7,
 25 for the implementation of a method for the identification of different populations or breeds of ruminant mammals such as cattle, sheep and goats.

9. Use of nucleotide primers according to claim 8, in the form of 5'-3' primer pairs, these pairs being such that:

30 - the 5' primer is chosen from the SIL10 primer represented by the following sequence SEQ ID NO: 10:

5' GTTGCTGGAAGGAAGAACAGGATGGATCTG 3'

or any sequence derived from this sequence SEQ ID NO: 10, in particular by suppression and/or substitution and/or addition of one or more nucleotides, said derived

sequence being hybridized, like the sequence SEQ ID NO: 10, with all or part of the nucleotide sequence complementary to the nucleotide sequences delimited by the nucleotides situated in positions 9 and 38 of the sequences SEQ ID NO: 1, 3, and 5,

- the 3' primer is chosen from the SIL8 primer represented by the following sequence SEQ ID NO: 11:

5' CAGTCCCAAGTGCCTGAACACACATGCACC 3'

or any sequence derived from this sequence SEQ ID NO: 11, in particular by suppression and/or substitution and/or addition of one or more nucleotides, said derived sequence being hybridized, like the sequence SEQ ID NO: 11, with all or part of the nucleotide sequences delimited by the nucleotides situated in positions 276 and 302 of the sequences SEQ ID NO: 1, 3, and by the nucleotides situated in positions 273 and 299, of the sequence SEQ ID NO: 5.

10. Nucleotide sequence characterized in that it corresponds to the bovine *SI* gene represented by SEQ ID NO:1, or to the following fragments of the *SI* gene:

- any fragment chosen from those of approximately 10 to 300 nucleotides containing the nucleotides situated in positions 82 to 93 of the sequence SEQ ID NO: 1,
- the fragment SEQ ID NO: 7 of 294 nucleotides delimited by the nucleotides situated in positions 9 and 302 of the sequence SEQ ID NO: 1.

11. Nucleotide sequence characterized in that it corresponds to the bovine *si* gene represented by SEQ ID NO: 3, or to the following fragments of the *si* gene:

- any fragment chosen from those of approximately 10 to 300 nucleotides containing the nucleotides situated in positions 82 to 93 of the sequence SEQ ID NO: 3,
- the fragment SEQ ID NO: 8 of 294 nucleotides delimited by the nucleotides situated in positions 9 and 302 of the sequence SEQ ID NO: 3.

12. Nucleotide sequence characterized in that it corresponds to the bovine *si₁* gene represented by SEQ ID NO: 5, or to the following fragments of the *si₁* gene:

- any fragment chosen from those of approximately 10 to 300 nucleotides containing the nucleotides situated in positions 82 to 93 of the sequence SEQ ID NO: 5,
- the fragment SEQ ID NO: 9 of 291 nucleotides delimited by the nucleotides situated in positions 9 and 299 of the sequence SEQ ID NO: 5.

13. Nucleotide sequence characterized in that it comprises:

- the SIL10 nucleotide sequence represented by the following sequence
SEQ ID NO: 10:

5' GTTGCTGGAAGGAAGAACAGGATGGATCTG 3'

or any sequence derived from this sequence SEQ ID NO: 10, in particular by suppression and/or substitution and/or addition of one or more nucleotides, said derived sequence being hybridized, like the sequence SEQ ID NO: 10, with all or part of the nucleotide sequence complementary to the nucleotide sequences delimited by the nucleotides situated in positions 9 and 38 of the sequences SEQ ID NO: 1, 3, and 5,

- the SIL8 nucleotide sequence represented by the following sequence
SEQ ID NO: 11:

5' CAGTCCCAAGTGCCTGAACACACATGCACC 3'

or any sequence derived from this sequence SEQ ID NO: 11, in particular by suppression and/or substitution and/or addition of one or more nucleotides, said derived sequence being hybridized, like the sequence SEQ ID NO: 11, with all or part of the nucleotide sequences delimited by the nucleotides situated in positions 276 and 302 of the sequences SEQ ID NO: 1, 3, and by the nucleotides situated in positions 273 and 299, of the sequence SEQ ID NO: 5.

14. Primer pairs, each of the two primers comprising, independently of one other, approximately 10 to approximately 30 nucleotides, characterized in that they are chosen in such a manner that one of the two sequences of a primer pair is hybridized with a sequence of approximately 10 to approximately 30 nucleotides comprised in the nucleotide sequence complementary to the sequence delimited by the nucleotides situated in positions 1 and approximately 60 of the nucleotide sequences SEQ ID NO: 1, 3, and 5, whilst the other sequence of this same pair is hybridized with a sequence of approximately 10 to approximately 30 nucleotides comprised between the nucleotide situated in position 94 and the last of the nucleotides of the sequences SEQ ID NO: 1, 3, and 5.

15. Primer pairs for gene amplification according to claim 10, characterized in that:

- one of the primers is chosen from the sequences comprising the SIL10 sequence represented by SEQ ID NO: 10, or any sequence derived from the latter, according to

claim 13, said primer being advantageously labelled, in particular in a radioactive or fluorescent manner,

- whilst the other primer is chosen from the sequences comprising the SIL8 sequence represented by SEQ ID NO: 11, or any sequence derived from the latter, according to claim 13.

16. Method for the identification of populations or breeds of ruminant mammals such as cattle, sheep and goats, said method being carried out starting with a biological sample collected from the animal, in particular starting with sperm, embryo, blood, milk, hairs, carcass or meat, or other products derived from the latter, and making it possible to check, or even to certify, whether or not the animal from which said biological sample was collected belongs to a population or breed of ruminant mammals, this method comprising:

- a stage of amplification of the number of copies of the different allelic forms of the *SILVER* gene, namely of the *SI*, and/or *si*, and/or *si₁* alleles, and/or of fragments of these allelic forms, specific to a population or breed of specific ruminant mammals, and capable of being present in said biological sample,

- a stage of detection of said allelic forms or fragments of the latter.

17. Identification method according to claim 16, characterized in that the stage of amplification of the number of copies of the different allelic forms of the *SILVER* gene, or of the fragments of these allelic forms, is carried out using a primer pair according to claim 14 or 15.

18. Identification method according to claim 16 or 17, characterized in that:

- the detection of a genotype comprising the *si* allele in the biological sample studied makes it possible to certify that said sample originates from an animal belonging to the Charolais breed or having at least one ancestor of the Charolais breed,

- the detection of a genotype comprising the *si₁* allele in the biological sample studied makes it possible to certify that said sample originates from an animal belonging to the Simmental breed or having at least one ancestor of the Simmental breed,

- the detection of a genotype comprising the *SI* allele, makes it possible to certify that said sample does not originate from an animal of the Charolais breed.

19. Kit for the implementation of a method according to one of claims 16 to 18, characterized in that it comprises at least one primer pair according to claim 14 or 15, and if appropriate the reagents necessary for the implementation of the amplification reaction of the number of copies of the different allelic forms of the *SILVER* gene.